

## WATER AND HEMOLYMPH CONTENT IN THE WOLF SPIDER *LYCOSA CERATIOLA* (ARANEAE, LYCOSIDAE)

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### ABSTRACT

Female *Lycosa ceratiola*, most of whom were gravid when collected in March in Florida, contained significantly less water than males (2.24 versus 2.88 mg water/mg dry mass, representing 69 and 74% of wet mass, respectively). Both sexes had similar amounts of hemolymph in their bodies (32.4% of wet mass in females and 37.3% in males). The density of hemolymph in male and female spiders at 22-24° C averaged 1.00 mg/ $\mu$ l. These results suggest that egg production in female spiders affects their total water content, most likely because ripening eggs gain energy-rich lipids at the expense of water. Two commonly used water content indices, which express water mass as a proportion of either wet or dry body mass, are evaluated.

### INTRODUCTION

Water and blood relations in spiders are poorly understood compared to information concerning insects, mites, and ticks. Moreover, the state of the field is heterogeneous: many basic physiological problems in spiders have attracted little attention, whereas a few topics, most notably hemolymph ionic and biological chemistry, have been well investigated (Pulz 1987; Strazny and Perry 1987; and references therein).

Here I attempt to resolve two apparently contradictory concepts underlying variability in water content in spiders (Pulz 1987). The first concept is that there is no consistent difference in water content between the sexes within a species. The second principle is that individual water content depends in part on lipid content, which is high in gravid females compared to males. I hypothesized that water content in gravid females should be significantly less than in males of a given species. Furthermore I hypothesized that the blood content of spiders might also show a similar sexual difference.

I here report on experiments with the wolf spider *Lycosa ceratiola* Gertsch and Wallace that test these ideas. In addition, I discuss the indices used to express water content in spiders. To my knowledge this is only the second study of hemolymph content in a spider. In this study I express water or hemolymph content as the proportion of spider body mass (Allen 1974).

## MATERIALS AND METHODS

Adult male and female *L. ceratiola* ( $N = 148$ ) were collected in March in xeric scrubby flatwoods at the Archbold Biological Station, Highlands County, Florida. At this time of year, as indicated by preliminary field surveys, reproduction is prevalent in this species (J. E. Carrel, unpublished observations). Spiders were maintained individually in plastic containers as described by Carrel and Eisner (1984). Their wet mass when alive was measured individually to the nearest 0.1 mg shortly before being used in tests. Individual spiders were used only in one test.

Water content of *L. ceratiola* was determined gravimetrically. Adult spiders ( $N =$  eight of each sex) were weighed, placed individually in a tared vial, killed by freezing, and then dried to constant mass in an 80° C oven. Water content was expressed as % wet mass and mg water/mg dry mass.

To calculate hemolymph content (% wet mass) I determined density and volume of hemolymph in spiders. Hemolymph density was measured in spiders ( $N =$  eight of each sex) as follows: individuals were anesthetized with carbon dioxide gas; a leg was amputated at the base; discharged hemolymph (2.7-11.1  $\mu$ l) was taken up in a volumetrically calibrated tube previously weighed to 1  $\mu$ g on a Cahn 28® electrobalance; the filled tube was reweighed and the volume of fluid in it was measured. Density of each hemolymph sample was calculated by dividing its volume by its mass (mg/ $\mu$ l).

Hemolymph volume in adults ( $N =$  eight of each sex) was determined using the radiolabeled inulin dilution method (Wharton et al. 1965). Injection (5  $\mu$ l) was accomplished with a micrometer syringe into the pericardial region of the abdomen of a spider anesthetized with carbon dioxide gas. Carboxy-14C-inulin (sp. act. 2.60 mCi/gram, Sigma Chemical Co.) was dissolved in spider saline (Rathmayer 1965) to achieve a dosage of 0.1  $\mu$ Ci per spider. Each spider was again anesthetized 1 h after injection and hemolymph was collected as previously described. The hemolymph was discharged immediately from the tube into 1 ml deionized water in a scintillation vial. Subsequently 15 ml of Aquasol scintillation fluid was added to each vial and radioactivity was measured in a Hewlett-Packard Tri-Carb 460C® scintillation counter. In a similar fashion the radioactivity in aliquots of the inulin stock solution was measured and used as a reference standard. To correct for counting inefficiencies and quenching effects, the sample channel ratio (SCR) was used to calculate total radioactivity (cpm) in each sample. Hemolymph volume of each spider was calculated as follows:

$$V_b = \frac{V_s C_i}{C_s} - V_i$$

where:  $V_b$  = volume of hemolymph in spider  
 $V_s$  = volume of hemolymph sample  
 $V_i$  = volume of solution injected (5  $\mu$ l)  
 $C_i$  = count of solution injected  
 $C_s$  = count of hemolymph sample

The reproductive state of female *L. ceratiola* ( $N = 100$ ) was determined in two ways. Using the method of Riddle (1985), 50 spiders were killed by freezing and their abdomens were bisected. Specimens with an egg mass greater than one-sixth of the cross-sectional area of the abdomen were considered gravid. To verify this

Table 1.—Dry mass and water content in adult *Lycosa ceratiola*. Differences between values with the same letter in a column are significant (a =  $P < 0.001$ ; b =  $P < 0.01$ ) with *t*-test. Mean  $\pm$  SE, (Range).

Sex	Dry mass mg	Water content		N
		% wet mass	mg water/mg dry mass	
Male	80.1 $\pm$ 8.9 <sup>a</sup>	74.0 $\pm$ 0.9 <sup>b</sup>	2.88 $\pm$ 0.14 <sup>b</sup>	8
	(48.3 - 117.2)	(71.3 - 78.2)	(2.48 - 3.58)	
Female	228.2 $\pm$ 26.4 <sup>a</sup>	69.0 $\pm$ 1.1 <sup>b</sup>	2.24 $\pm$ 0.10 <sup>b</sup>	8
	(157.5 - 388.4)	(61.5 - 71.2)	(1.59 - 2.47)	

procedure, the remaining 50 spiders were inspected at 2-3 day intervals for 4 wk to ascertain whether each had produced an egg sac.

Statistical analyses of the data were performed manually using the methods described in Sokal and Rohlf (1987) or by computer using SAS routines (SAS 1985).

## RESULTS AND DISCUSSION

Living adult *L. ceratiola* exhibited a sexual size dimorphism. Data ( $\bar{X} \pm$  SE,  $N = 24$  of each sex) showed female and male spiders weighed  $724 \pm 56$  and  $305 \pm 29$  mg, respectively. This difference, a factor equal approximately to 2.37, was highly significant (*t*-test,  $P < 0.001$ ). Female spiders are larger than males, presumably because females invest much more in reproduction than males (Gertsch 1979; Foelix 1982). There was no significant difference (ANOVA,  $P > 0.01$ ) in wet body mass among spiders used in different experiments.

Female *L. ceratiola* contained proportionately more dry mass and, therefore, less water than males (Table 1). By either index used in Table 1, water content in female spiders was significantly less than in males. The female/male dry mass ratio was 2.85, approximately 20% higher than the wet mass ratio.

Whole body water content in *L. ceratiola* was slightly less than generally reported for adult spiders from a variety of biomes in North America (Stewart and Martin 1970; Vollmer and MacMahon 1974; Riddle 1985). In all of these studies the spiders were well watered in the laboratory, so dehydration should not be a significant factor. Moreover, Vollmer and MacMahon (1974) found no correlation between habitat aridity, body mass, and interspecific differences in water content of spiders. Surely the relationship between water content and physiological ecology in spiders is sufficiently complex that many more data from many more species are needed to discern life history patterns.

Density and relative amount of hemolymph was similar in male and female *L. ceratiola* (Table 2). Females contained relatively less hemolymph than males, but because of the variability in the data, the difference between the sexes was not significant (*t*-test,  $P > 0.05$ ). Whether the high degree of intrasexual variability in hemolymph content is biologically meaningful or the result of an artifact remains to be determined.

To my knowledge this is the first report of using dilution of radiolabeled inulin injected into spiders. Stewart and Martin (1970), using unlabeled inulin as a blood-borne dye, reported the hemolymph in male and female *Dugesia hertzi*

Table 2.—Hemolymph density and content in adult *L. ceratiola*. Differences between values in the same column are not significant ( $P > 0.1$ ) with *t*-test. Mean  $\pm$  SE, (Range).

Sex	Hemolymph density mg/ $\mu$ l	Hemolymph content % wet mass	N
Male	1.003 $\pm$ 0.007 (0.97 - 1.03)	37.3 $\pm$ 2.4 (28.2 - 46.9)	8
Female	1.000 $\pm$ 0.005 (0.98 - 1.02)	32.4 $\pm$ 6.5 (26.6 - 41.6)	8

averages 19.65 and 18.10%, respectively, of wet body mass. Although the hemolymph content in *D. hentzi* adults is about one-half as much as in *L. ceratiola*, a difference which in part could result from using different methodologies, nevertheless within each species females tend to have less hemolymph than males.

A majority (74%) of 50 female *L. ceratiola* examined internally were found to be gravid. This method was verified by the finding that a smaller, but insignificantly different percentage (58%) of 50 females actually produced egg sacs when maintained for 4 wk in the laboratory (chi-square test,  $P > 0.05$ ). Hence, lipid content of female spiders used in these water and blood content studies presumably was high because most of them contained energy rich eggs. The energy density of spider eggs, expressed as joules/g dry mass, generally is 11% higher than the average for nongravid adult spiders (Anderson 1978).

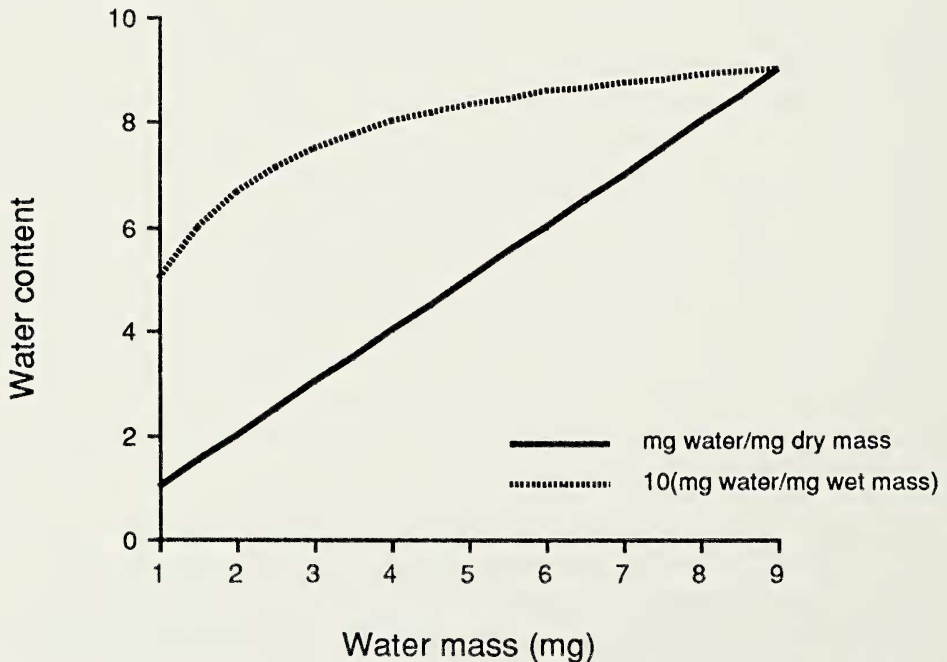


Figure 1.—Comparison of two indices for water content in a hypothetical spider having dry mass of 1 mg as a function of its absolute water mass. (See text for details). The range of water content values matches those actually found in various spiders under different conditions, as summarized by Pulz (1987). For graphical purposes, water content based on wet body mass is shown at one-tenth scale so that the two lines are similar in slope.

As indicated in Table 1, the water content of whole spiders can be expressed by two different indices, one based on the wet mass and the other based on the dry mass of the animal. Most authors, as cited by Pulz (1987), have used the wet mass index, often referred to as "percent water". But is one index scientifically more robust than the other? One way to answer this question is to examine how body water content changes as a function of water mass in a spider, under idealized conditions where dry mass is kept constant (say equal to 1 mg) as if the animal is undergoing dehydration or rehydration. As shown in Fig. 1, under these hypothetical conditions the two indices yield two rather different graphs: the wet mass index levels off asymptotically as the spider gains a lot of water, whereas the dry mass index rises in a linear fashion across the same range.

From this graphical analysis, clearly the linear dry mass index is preferable to the curvilinear wet mass index of body water content. An example will illustrate this conclusion. At high moisture levels, a one percent gain or loss in water content based on a spider's wet body mass translates into a large change approximating 1 mg water/mg dry mass of the animal.

In conclusion, this study shows that a consistent difference in water content between the sexes of *L. ceratiola* can be found when females are gravid. The presence of eggs evidently increases the lipid and dry mass contents in female spiders, causing a slight (5%) decline in water content in comparison to male spiders.

#### ACKNOWLEDGMENTS

I thank Z. Yang and M. H. McCairel for field and laboratory assistance in preliminary studies, M. Deyrup and the staff of the Archbold Biological Station for hospitality and research facilities, J. D. David and G. H. Perrot for technical assistance, and J. F. Anderson and K. N. Prestwich for reviewing the manuscript. Supported in part by Research Incentive Funds from the University of Missouri-Columbia.

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*Manuscript received May 1989, revised June 1989.*